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Synthesis of Chiral Isoxazolidin-5-ones and their Applications to the Synthesis of β-Amino-Alanines and β-(N-Hydroxyamino)-Alanines

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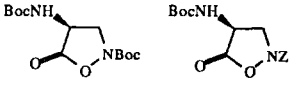
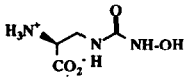
Abstract: Herein we report a high-yielding synthesis of isoxazolidin-5-ones and their use to synthesise both β-amino-alanines and β-(N-hydroxyamino)-alanines.

Functionalised β-amino-alanines represent an important class of naturally occurring non-proteinogenic amino acids[1]. Whilst a retrosynthetic analysis would suggest a synthesis by reaction of a nitrogen-based nucleophile with an alanine fragment bearing a suitable leaving group in the β-position, attempts to synthesise β-amino-alanines by such methods often results in optically impure material as a result of β-elimination followed by readdition of the nucleophile in a conjugate manner[2,3]. Thus syntheses must be designed in such a way as to minimise β-elimination. One method of achieving this is to carry out the β-substitution in an intramolecular sense. This method has been used successfully to synthesise β-amino alanines *via* β-lactams[4] and isoxazolidin-5-ones[5,6]. In this paper we report an improved synthesis of chiral isoxazolidin-5-ones (1) and (2), and their use to synthesise biologically important β-functionalised alanines such as β-(3-hydroxyureido)-alanine (3) and β-(isoxazolin-5-one-2-yl)-alanine (4).



(1) (2)



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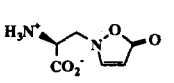
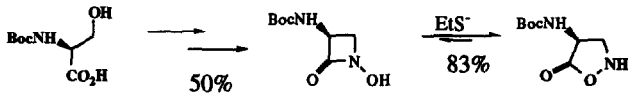


(4)

Synthesis of isoxazolidin-5-ones (1) and (2)

Substituted isoxazolidin-5-ones (eg. (7)), have previously been synthesised by thiol-mediated isomerisation of β-lactams in 42% overall yield from N-*tert*-butoxycarbonyl-L-serine[5,6] (scheme 1).



(5) (6) (7)

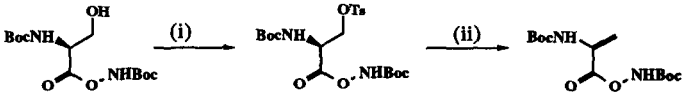
Scheme 1

We believed that a synthesis by intramolecular displacement of a β-substituted alanine to form a 5-membered ring would be a more efficient procedure, since this would avoid the need to proceed *via* a β-lactam, and would thus give a higher yield of substituted isoxazolidin-5-one (scheme 2).

(8) (9)

Scheme 2

Attempts to cyclise (10) using either DEAD/PPh_3 or CCl_4/PPh_3/ Et_3N at a variety of temperatures proved unsuccessful, whilst attempted tosylate displacement of (11) using triethylamine as a base resulted in β-elimination (scheme 3).

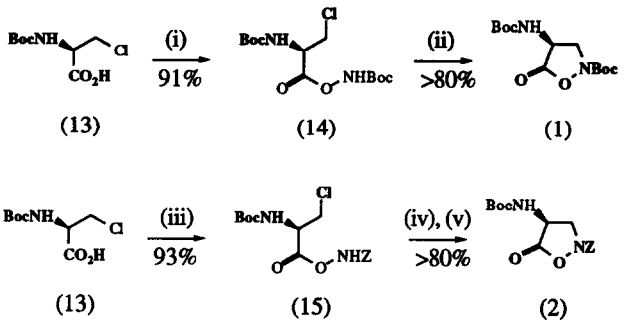


(10) (11) (12)

(i) TsCl, pyridine, CH_2Cl_2; (ii) Et_3N

Scheme 3

Subsequently, displacement of chloride was attempted: N-*tert*-butoxycarbonyl-β-chloroalanine (13) was synthesised from serine in 76% yield, using standard procedures[7]. This was coupled with N-*tert*-butoxycarbonyl-hydroxylamine to provide (14) in 91% yield, using EDCI as the coupling agent. This compound was successfully cyclised using sodium hydride in dimethylformamide at high dilution at 0°C in >80% yield (lower dilution resulted in substantial β-elimination). The resulting isoxazolidin-5-one (1) was labile, and was not purified, but used directly in proceding reactions. Isoxazolidin-5-one (2) was synthesised likewise from N-benzyloxycarbonyl-hydroxylamine and β-chloroalanine (in addition, it was found that initial addition of sodium iodide (1 eq.) to the cyclisation reaction minimised β-elimination) (scheme 4).



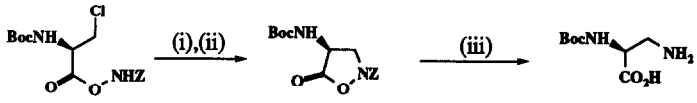
(i) BocNHOH, EDCI, CH_2Cl_2; (ii) NaH, DMF, 0°C; (iii) ZNHOH, EDCI, CH_2Cl_2; (iv) NaI, DMF, 0°C; (v) NaH, DMF, 0°C-room temp.

Scheme 4

In this way substituted isoxazolidin-5-ones (1) and (2) were prepared in 6 steps from L-serine in approximately 55% yield.

Synthesis of β-amino alanines

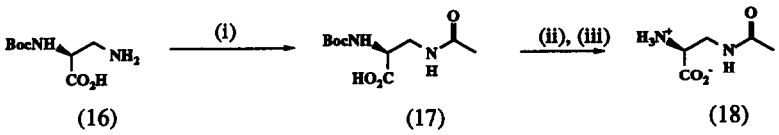
We reasoned that hydrogenation of isoxazolidin-5-one (2) would provide α-N-*tert*-butoxycarbonyl-β-amino alanine (16), a useful selectively protected reagent for the synthesis of β-amino alanines. Thus hydrogenation using Pd/C catalyst, in methanol (not anhydrous), gave protected amino acid (16) in 55% overall yield from β-chloroalanine derivative (15) *via* isoxazolidin-5-one (2) (scheme 5).



(i) NaI, DMF, 0°C; (ii) NaH, DMF, 0°C-room temp.; (iii) H_2, Pd/C, MeOH

Scheme 5

In order to determine the optical purity of the α-N-*tert*-butoxycarbonyl-β-amino alanine, it was carried through to form both the well-characterised β-acetylamino-alanine (18), and the S- and R-mandeloyl derivatives (20a and 20b) of this compound. Thus firstly, α-N-*tert*-butoxycarbonyl-β-amino alanine (16) was treated with acetic anhydride and pyridine. Subsequent deprotection using trifluoroacetic acid, followed by purification by ion-exchange chromatography (Dowex 50W-X8 resin (100-200 mesh), eluting with 2N ammonium hydroxide), resulted in β-acetylamino-alanine, $[\alpha_D]^{20}$ -85.7 (c 1.75, H_2O)(cf lit.[8] -87 (c 8, H_2O)) (scheme 6).

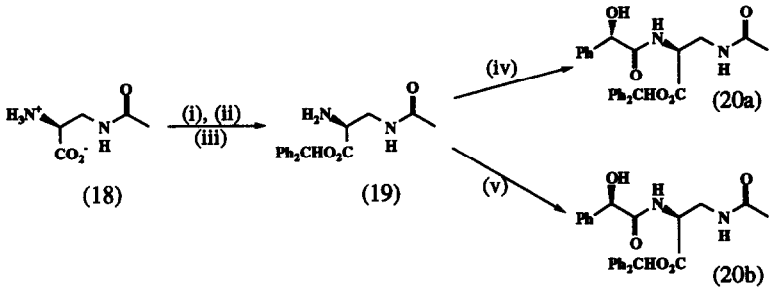


(16) (17) (18)

(i) Ac_2O, pyridine, CH_2Cl_2; (ii) CF_3CO_2H; (iii) ion exchange

Scheme 6

Secondly, β-acetylamino-alanine was treated with *p*-toluenesulphonic acid in aqueous acetonitrile, then diphenyldiazomethane. The resulting salt was basified, extracted, and coupled separately with S-and R-mandelic acid to provide separate diastereomers (20a and 20b), which were distinguishable by ^1H nmr, thus confirming the optical integrity of the β-amino acid (16) (scheme 7).

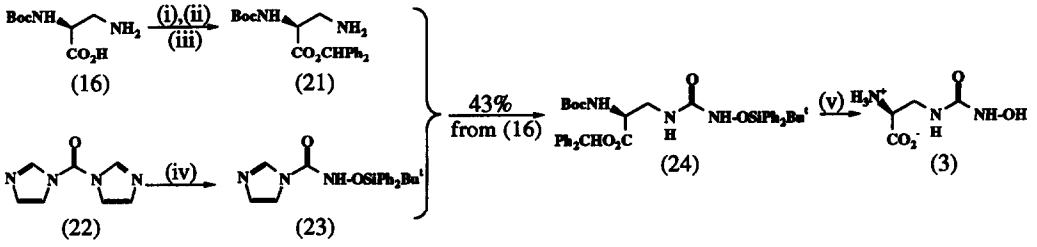


(18) (19) (20a) (20b)

(i) TsOH, CH_3CN/H_2O; (ii) Ph_2CN_2; (iii) $NaHCO_3$, H_2O/EtOAc;
(iv) (S)-Mandelic acid, EEDQ, CH_2Cl_2; (v) (R)-Mandelic acid, EEDQ, CH_2Cl_2

Scheme 7

In order to demonstrate the application of this procedure for the synthesis of β-amino alanines, we synthesised β-(3-hydroxyureido)-alanine (3), an antibiotic amino acid isolated from *Streptomyces hygroscopicus*[9], and previously synthesised by Takemoto[10] in low yield from aspartic acid, with unstated optical purity. We designed a synthesis based upon α-N-*tert*-butoxycarbonyl-β-amino alanine benzhydryl ester (21), using carbonyl diimidazole (22), and a suitable O-protected hydroxylamine derivative. Thus α-N-*tert*-butoxycarbonyl-β-amino alanine (16) was esterified by treatment with *p*-toluenesulphonic acid then diphenyldiazomethane, followed by basic extraction. The resultant amine (21) was then added to the product of slow addition of O-*tert*-butyldiphenylsilyl hydroxylamine to carbonyl diimidazole (22), in dichloromethane, and the resulting ureido-compound (24) was purified by chromatography. Deprotection using dilute HCl, followed by purification by ion-exchange chromatography (Dowex 50W-X8 resin (100-200 mesh), eluting with 2N ammonium hydroxide) gave β-(3-hydroxyureido)-alanine (3) with $[\alpha_D]^{20}$ -11.5 (c 0.5, 1N HCl) (cf lit.[9] -12 (c 1.0, 1N HCl)), in 18% yield from α-N-*tert*-butoxycarbonyl-β-amino alanine (16) (7% from L-serine) (scheme 8).

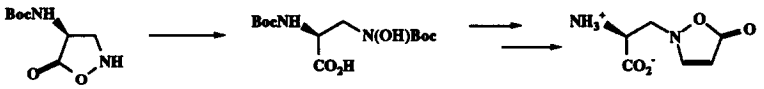


(i) TsOH, CH_3CN/H_2O; (ii) Ph_2CN_2; (iii) $NaHCO_3$, H_2O/EtOAc;
(iv) $Bu^tPh_2SiONH_2$, CH_2Cl_2; (v) dil. HCl, dioxan/H_2O

Scheme 8

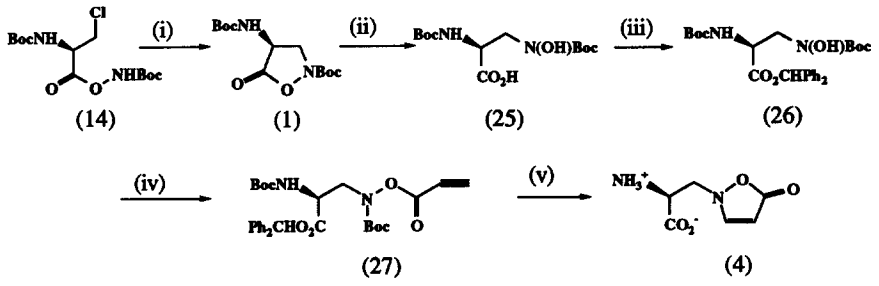
Synthesis of β-(N-hydroxyamino)-alanines

In connection with studies of biosynthetic pathways in *Lathyrus sativus*, we required to synthesise isotopically labelled forms of β-(isoxazolin-5-one-2-yl)-alanine (BIA) (4). BIA has previously been synthesised[6] from isoxazolidin-5-one (7) *via* N-hydroxy-acid (25) (scheme 9).



Scheme 9

We reasoned that we could synthesise N-hydroxy-acid (25) from isoxazolidin-5-one (1), and thus improve on the yield of BIA from serine, and, more importantly, from hydroxylamine (an important feature concerning a synthesis of ^{15}N-BIA), compared with the published synthesis. Thus isoxazolidin-5-one (1) was hydrolysed using caesium carbonate in aqueous THF, to give N-hydroxy-acid (25). This was not purified, but derivatised directly as the benzhydryl ester by treatment with diphenyldiazomethane in acetonitrile. Chromatographic purification of this compound resulted in an overall 84% yield from β-chloroalanine derivative (14). This ester was coupled with propiolic acid (1.6 eq.) in 91% yield, using DCC as the coupling agent, and the resulting ester (27) was deprotected and cyclised *in situ* by treatment with 98% formic acid[6] (scheme 10).

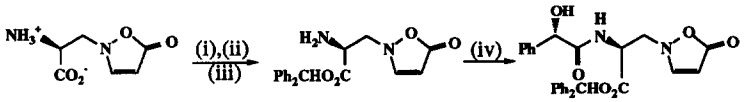


(14) (1) (25) (26) (27) (4)

(i) NaH, DMF, 0°C; (ii) Cs_2CO_3, H_2O/DMF, then dil. HCl/ EtOAc extraction; (iii) Ph_2CN_2, CH_3CN; (iv) $HC{\equiv}CO_2H$ (1.6 eq.), DCC, CH_2Cl_2; (v) HCO_2H, 35°C, 18 hours

Scheme 10

This synthesis resulted in a 45% overall yield of BIA from serine (cf published synthesis[6], *ca* 10% yield from serine). In order to asses the optical purity of the BIA, it was derivatised as its S mandeloyl derivative (29)[11]. Thus BIA was treated with *p*-toluenesulphonic acid in aqueous acetonitrile, then diphenyldiazomethane. The resulting salt was basified to produce amine (28), which was coupled with S-mandelic acid. The resulting dipeptide was purified by preparative plate chromatography, taking care to combine all fractions containing BIA. Analysis by nmr showed the presence of two compounds, corresponding to derivatives of L-BIA and D-BIA in relative proportions of 9:1 (scheme 11). Thus the synthesis resulted in BIA with 80% enantiomeric excess (cf published synthesis[6], 90% ee).

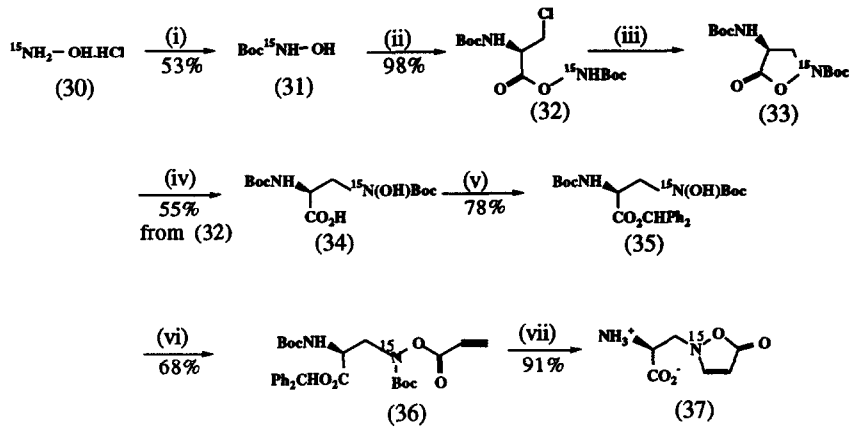


(4) (28) (29)

(i) TsOH, acetone/H_2O; (ii) Ph_2CN_2; (iii) $NaHCO_3$, H_2O/EtOAc; (iv) (S)-Mandelic acid, EEDQ, CH_2Cl_2

Scheme 11

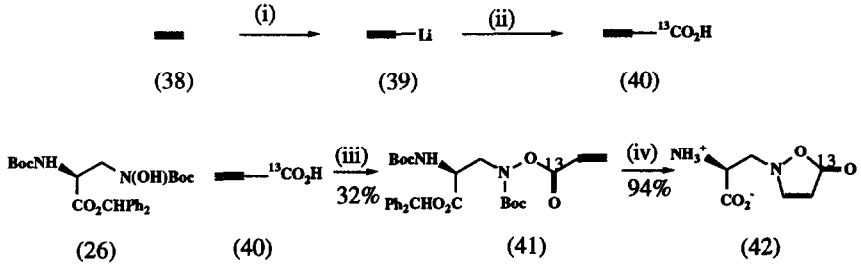
Despite slightly lower optical efficiency, this synthesis was suitable for the synthesis of β-^{15}N-labelled BIA, as the yield of BIA was 43% with respect to hydroxylamine hydrochloride [cf published synthesis[6] (which would require low-yielding O-benzyl protection of hydroxylamine hydrochloride) in approximately 5% overall yield from hydroxylamine hydrochloride]. Thus ^{15}N-hydroxylamine hydrochloride was treated with di-*tert*-butyl-dicarbonate in aqueous alkaline dioxan to form ^{15}N-*tert*-butoxycarbonyl-hydroxylamine in 53% yield. This was carried through the synthesis, as descibed for the synthesis of unlabelled BIA, to form in practice β-^{15}N-labelled BIA (37) in 14% overall yield from ^{15}N-hydroxylamine hydrochloride (scheme 12).



(i) Boc_2O, NaOH, dioxan/H_2O; (ii) Boc-β-chloro-alanine (13), EDCI, CH_2Cl_2; (iii) NaH, DMF, 0°C;
(iv) Cs_2CO_3, H_2O/DMF, then dil. HCl/EtOAc extraction; (v) Ph_2CN_2, CH_3CN;
(vi) $HC{\equiv}CCO_2H$ (1.1 eq.), DCC, CH_2Cl_2; (vii) HCO_2H, 35°C, 15 hours

Scheme 12

A synthesis of BIA with a ^{13}C label in the carbonyl group of the isoxazolinone ring was likewise achieved by coupling of N-hydroxy-ester (26) with 1-^{13}C-propiolic acid. Thus 1-^{13}C-propiolic acid was synthesised (in low yield) by treatment of acetylene with butyllithium, followed by quenching with $^{13}CO_2$. This acid (40) could not be readily purified, thus it was coupled directly with N-hydroxy-ester (26) in 32% yield, and the resulting ester (41) was carried through the synthesis as described above, to form ^{13}C-BIA (42) (scheme 13).



(i) BuLi, THF; (ii) $^{13}CO_2$; (iii) DCC, CH_2Cl_2; (iv) HCO_2H, 35°C, 15 hours

Scheme 13

In summary, we have demonstrated a new synthesis of chiral isoxazolidin-5-ones, and have applied this methodology to the synthesis of both functionalised β-amino-alanines and β-(N-hydroxyamino)-alanines.

Syntheses of labelled compounds have also been demonstrated, and the results of biological experiments on these compounds [(37) and (42)] will be reported elsewhere.

Experimental Section

Melting points were determined using a Büchi 510 capillary melting point apparatus, and are quoted uncorrected; optical rotations were measured with a Perkin-Elmer 241 polarimeter at 20°C with a pathlength of 1 dm; concentrations (c) are reported in g/100 ml. Microanalyses were performed within the Dyson Perrins Laboratory. Infra-red spectra were recorded on a Perkin-Elmer 1750 FT spectrometer (absorptions are quoted in cm^{-1}). NMR spectra were recorded using Varian Gemini 200 and Bruker AM500 spectrometers; chemical shifts (δ_H) are quoted in parts per million (ppm) and are referenced to residual protonated solvent resonances; coupling constants (*J*) were recorded to the nearest 0.5 Hz. ^{13}C NMR spectra were recorded using a Varian Gemini 200 operating at 50.3 MHz. Carbon multiplicities were determined by operating the spectrometers in DEPT mode. ^{15}N magnetic resonance spectra were recorded using a Bruker AM250 spectrometer operating at 25.3 MHz, and were referenced externally to neat $Me^{15}NO_2$ at 0 ppm. Mass spectra were recorded on the following instruments: V.G. Micromass ZAB 1F (FAB/CI/DCI); V.G.Masslab 20-250 (CI/DCI); V.G. BIO-Q (electrospray).

Thin-layer chromatography was performed on Merck kieselgel DC-Alufolien $60F_{254}$ 0.2 mm precoated plates, visualisation was by quenching of uv fluorescence, or 5% w/v dodecamolybdophosphoric acid in ethanol. Preparative-layer chromatography was carried out on silica gel (HF254-Blend 41) coated to 1 mm thickness on 20 x 20 cm glass plates. Flash chromatography was carried out on Baker silica gel (30-60 μm). Ion exchange was carried out on Dowex 50W-X8(H) resin which was charged using 2N HCl (aq.), then washed with water. High performance liquid chromatography (HPLC) was performed on a Waters 600E Multisolvent Delivery System, a Rheodyne 7125 injector, a Waters 991 Photodiode Array Detector, and a column packed with Hypersil (ODS 250 x 7 mm diameter).

All solvents were distilled before use; THF was distilled from sodium benzophenone ketyl under nitrogen; CH_2Cl_2 and DMF were distilled from CaH_2 under argon, and stored over activated 4Å molecular sieves; diphenyldiazomethane was prepared by oxidation of benzophenone hydrazone using HgO[12].

Reactions were carried out at room temperature, under an atmosphere of argon, unless otherwise stated.

1-*O*-(Benzyloxycarbamoyl)-(*N*-*tert*-butoxycarbonyl)-β-chloro-L-alanine (15): 1-Ethyl-3-[3-(dimethylamino)propyl]-carbodiimide (EDCI) (0.350 g, 1.8 mmol) was added to a stirred solution of *N*-(benzyloxycarbonyl)-hydroxylamine (prepared by the method of Boyland[13])(0.252 g, 1.5 mmol) and *N*-(*tert*-butoxycarbonyl)-β-chloro-L-alanine (13) (prepared by the method of Walsh[7]) (0.337 g, 1.5 mmol) in anhydrous dichloromethane (20 ml) at 0°C and the solution was stirred for 15 minutes, warmed to room temperature, and stirred for 15 hours. The dichloromethane was evaporated *in vacuo* and the resulting oil dissolved in ethyl acetate (10 ml), washed with water (2 x 10 ml), dried (Na_2SO_4), and the ethyl acetate evaporated *in vacuo* to give the title compound as a colourless oil (0.520 g, 93%); R_f 0.2 (20% ethyl acetate / petroleum ether (b.p.30-40°C)); ν_{max} ($CHCl_3$) 3 438 (m), 3 345 (br, w), 2 983 (m), 2 258 (m), 1 798 (s), 1 760 (s), 1 718 (s), 1 500 (s), 1 456 (m), 1 394 (m), 1 371 (s), 1 342 (m), 1 250 (s), 1 160 (s), 1 047 (m); δ_H (200 MHz, $CDCl_3$) 1.47 (9H, s, $(CH_3)_3C$-), 3.83-3.99 (2H, AB-X, *J* 3.0, 4.0, 11.5 Hz, -CH_2CH-), 4.87-4.92

(1H, AB-X, -CH_2C<u>H</u>-), 5.24 (2H, s, PhCH_2-), 5.43 (1H, d, *J* 8.5 Hz, -CHN<u>H</u>-), 7.39 (5H, s, aromatic CH), 8.26 (1H, s, -ONH); δ_C (50.3 MHz, $CDCl_3$) 28.1 ((<u>C</u>H_3)$_3$C-), 44.6 (-<u>C</u>H_2CH-), 53.3 (-CH_2<u>C</u>H-), 68.5 (Ph<u>C</u>H_2-), 81.1 ((CH_3)$_3$<u>C</u>-), 128.5-128.8 (aromatic CH), 135.1 (aromatic *ipso*), 155.3 and 156.5 (2 x -NH-<u>C</u>O_2C-), 169.0 (-CH-<u>C</u>O_2C-); *m/z* (CI (NH_3)) 392 (MNH_4^+, 10%), 390 (MNH_4^+, 22), 355 (16), 354 (73), 334 (57), 298 (100), 185 (51), 169 (54), 149 (58), 108 (100), 106 (73), 91 (62).

<u>S</u>-2-Benzyloxycarbonyl-4-[(*tert*-butoxycarbonyl)amino]-isoxazolidin-5-one (2): NaI (0.083 g, 0.55 mmol) was added to a stirred solution of 1-*O*-(benzyloxycarbamoyl)-*N*-*tert*-butoxycarbonyl-β-chloro-<u>L</u>-alanine (15) (0.205 g, 0.55 mmol) in anhydrous dimethylformamide (10 ml) at 0°C and the suspension was stirred for 30 minutes. A further 40 ml of dimethylformamide was added at 0°C, followed by NaH (0.017 g of a 60% dispersion in mineral oil, 0.8 eq.). The solution was stirred at 0°C for 15 minutes, then at room temperature for 90 minutes, before water (50 ml) was added, and the solution stirred for 10 minutes. It was then extracted with ethyl acetate (5 x 20 ml) and the organic layers were combined, washed with water (3 x 30 ml) and dil. HCl (2 x 20 ml), dried (Na_2SO_4), and the ethyl acetate evaporated *in vacuo* to give the crude title compound as a colourless oil (0.185 g). This material was greater than 80% pure by nmr and was used directly in proceeding reactions, without further purification; ν_{max} ($CHCl_3$) 3 421 (w), 3 025 (w), 1 812 (m), 1 724 (s), 1 500 (m), 1 456 (w), 1 371 (w), 1 155 (s); δ_H (200 MHz, $CDCl_3$) 1.45 (9H, s, ((CH_3)$_3$C-), 3.89-3.95 (1H, m, -CH_2C<u>H</u>-), 4.55-4.75 (2H, m, -C<u>H</u>$_2$CH-), 5.31 (2H, s, PhC<u>H</u>$_2$-), 7.40 (5H, s, aromatic CH); δ_C (50.3 MHz, $CDCl_3$) 28.1 ((<u>C</u>H_3)$_3$C-), 50.0 (-CH_2<u>C</u>H-), 53.1 (-<u>C</u>H_2CH-), 68.6 (Ph<u>C</u>H_2-), 81.4 ((CH_3)$_3$<u>C</u>-), 128.5-128.8 (aromatic CH), 135.0 (aromatic *ipso*), 155.4 and 156.8 (2 x -NH-<u>C</u>O_2C-), 171.6 (-CH-<u>C</u>O_2-N); *m/z* (CI (NH_3)) 355 (12%), 354 (MNH_4^+, 41), 298 (58), 237 (27), 193 (21), 108 (53), 91 (100).

α-*N*-*tert*-Butoxycarbonyl-β-amino-<u>L</u>-alanine (16)[14]: <u>S</u>-2-Benzyloxycarbonyl-4-[(*tert*-butoxycarbonyl)amino]-isoxazolidin-5-one (2) (0.420 g) was dissolved in methanol (25 ml), and 10% Pd/C (0.02 g) was added. The flask was purged, firstly with argon, then twice with hydrogen, and the suspension was stirred vigorously for 24 hours. The catalyst was removed by filtration through a bed of celite, and the methanol was evaporated *in vacuo*. The resulting white solid was dissolved in water (25 ml) and washed with ethyl acetate (2 x 25 ml). The aqueous fraction was freeze dried, to give the title compound as a white solid (0.139 g, 55% from (15)). Recrystallisation from water / ethanol gave an analytical sample; m.p. 184-186°C (lit.[14] 198-200°C (decomp.); $[\alpha]_D^{20}$ -16.5 (c 3, H_2O); δ_H (200 MHz, D_2O) 1.20 (9H, s, (CH_3)$_3$C-), 2.92-3.01 and 3.12-3.27 (2H, <u>AB</u>-X, *J* 13.0, 7.0, 6.5 Hz, -C<u>H</u>$_2$CH-), 3.87-4.01 (1H, m, -CH_2C<u>H</u>-); δ_C (125 MHz, D_2O) 28.3 ((<u>C</u>H_3)$_3$C-), 41.9 (-CH_2-), 53.6 (-CH-), 82.4 ((CH_3)$_3$<u>C</u>-), 158.1 (-NH-CO_2-), 175.2 (-CH<u>C</u>O_2-); *m/z* (DCI (NH_3)) 205 (MH^+, 64%), 149 (100).

α-*N*-*tert*-Butoxycarbonyl-β-acetylamino-<u>L</u>-alanine (17): Pyridine (0.065 ml, 1.1 eq.) and acetic anhydride (0.08 ml, 1.2 eq.) were added to a stirred suspension of α-*N*-*tert*-butoxycarbonyl-β-amino-<u>L</u>-alanine (16) (0.150 g, 0.74 mmol) in anhydrous dichloromethane (10 ml), and the reaction mixture was stirred for 24 hours to give a yellow solution. The dichloromethane was evaporated *in vacuo*, and the resulting yellow oil was dissolved in ethyl acetate (10 ml) and washed with dil. HCl (10 ml). The ethyl acetate layer was dried (Na_2SO_4), and the ethyl acetate evaporated *in vacuo* to give the title compound as a yellow oil (0.152 g, 84%). This was used directly in the proceeding reaction without further purification; ν_{max} ($CHCl_3$) 3 000 (s), 2 980 (m),

1 700 (s), 1 670 (s), 1 520 (m), 1 500 (m), 1 365 (m); δ_H (200 MHz, $CDCl_3$) 1.45 (9H, s, $(CH_3)_3C$-), 2.05 (3H, s, CH_3CO-), 3.65-3.78 (2H, m, -CH_2CH-), 4.28-4.32 (1H, m, -CH_2CH-), 6.0 (1H, d, *J* 8.0 Hz, -CHNH-), 6.8 (1H, br, -CH_2NH-), 7.8 (1H, br, -CO_2H); δ_C (50.3 MHz, $CDCl_3$) 22.5 (CH_3CO-), 28.1 (($CH_3)_3$C-), 41.8 (-CH_2CH-), 54.2 (-CH_2CH-), 80.2 (($CH_3)_3$C-), 156.4 (-NH-CO_2-), 173.3 (-CHCO_2-), 185.4 (-NH$COCH_3$); *m/z* (CI (NH_3) 247 (MH^+, 12%), 191 (58), 147 (100).

β-acetylamino-L-alanine (18)[15,8]: α-*N*-*tert*-Butoxycarbonyl-β-acetylamino-L-alanine (17) (0.152 g, 0.62 mmol) was dissolved in trifluoroacetic acid (1 ml) and the solution was stirred for 1 hour. The trifluoroacetic acid was evaporated *in vacuo* and the resulting oil dissolved in water (3 ml) and washed with diethyl ether (2 x 3 ml). The aqueous layer was freeze dried to give β-acetylamino-L-alanine trifluoroacetate (0.100 g). This was purified by ion exchange chromatography, using Dowex 50W-X8 resin (100-200 mesh), eluting with 2N ammonium hydroxide, to give the title compound as a white solid (0.040 g, 44% from α-*N*-*tert*-butoxycarbonyl-β-acetylamino-L-alanine (17)); m.p. 168-172°C (lit.[15] 175-178°C); $[\alpha]_D^{20}$ -85.7 (c 1.75, H_2O) (lit.[8] -87 (c 8, H_2O)); δ_H (200 MHz, D_2O) 1.86 (3H, s, CH_3CO-), 3.39-3.44 and 3.60-3.64 (2H, AB-X, *J* 15.0, 7.5, 4.0 Hz, -CH_2CH-), 3.72-3.76 (1H, AB-X, -CH_2CH-); δ_C (62.9 MHz, D_2O) 22.5 (CH_3CO-), 40.8 (-CH_2CH-), 55.9 (-CH_2CH-), 173.3 and 176.3 (-C=O-); *m/z* (electrospray) 147 (MH^+, 100%).

β-acetylamino-L-alanine benzhydryl ester (19): β-Acetylamino-L-alanine (18) (0.040 g, 0.27 mmol) and *p*-toluene sulphonic acid (0.057g, 1.1 eq.) were dissolved in acetonitrile / water (2:1) (10 ml), and the solution was stirred for 10 minutes. Diphenyldiazomethane (0.074 g, 1.4 eq.) was then added, and the resulting solution was stirred for 1 hour, during which time the solution was decolourised. The acetonitrile was evaporated *in vacuo*, to give a white suspension. Dil. HCl (3 ml) was added, and the aqueous solution washed with diethyl ether (2 x 5 ml). The ether layers were re-extracted with dil. HCl (2 x 3 ml), and the aqueous layers combined, basified with saturated $NaHCO_3$, and extracted with ethyl acetate (5 x 10 ml). The ethyl acetate layers were combined, dried (Na_2SO_4), and the ethyl acetate evaporated *in vacuo* to give the title compound as a white solid (0.032 g, 38%); R_f 0.2 (ethyl acetate); ν_{max} ($CHCl_3$) 3 156 (w), 2 929 (w), 2 254 (s), 1 736 (s), 1 678 (m), 1 495 (w), 1 455 (m), 1 381 (s), 1 288 (m); δ_H (200 MHz, $CDCl_3$) 1.92 (3H, s, CH_3CO-), 2.5 (2H, br, -NH_2), 3.41 (1H, br, -CH_2CH-), 3.68-3.81 (2H, br, -CH_2CH-), 6.03 (1H, m, -CH_2NH-), 6.90 (1H, s, Ph_2CH-), 7.28-7.60 (10H, m, aromatic CH); *m/z* (DCI (NH_3) 313 (MH^+, 22%), 167 (100).

α-S-Mandeloyl-β-acetylamino-L-alanine benzhydryl ester (20a): Freshly prepared β-acetylamino-L-alanine benzhydryl ester (19) (0.011g, 0.035 mmol), S-mandelic acid (0.006 g, 1.1 eq.), and 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) (0.010 g, 1.2 eq.) were dissolved in anhydrous dichloromethane (2 ml), and the solution was stirred for 18 hours. The dichloromethane was evaporated *in vacuo*, and the resulting oil was dissolved in ethyl acetate (5 ml), and washed with dil. HCl (5 ml), saturated $NaHCO_3$ (5 ml) and water (5 ml). The ethyl acetate solution was dried (Na_2SO_4), and concentrated *in vacuo*, to give the crude title compound (0.011 g, 70%). This was purified by preparative plate flash chromatography, eluting with 20% ethyl acetate / petroleum ether (b.p. 30-40°C), to give the title compound (0.002 g, 13%); R_f 0.4 (20% ethyl acetate / petroleum ether (b.p. 30-40°C)); δ_H (200 MHz, $CDCl_3$) 1.70 (1H, s, -OH), 1.82 (3H, m, CH_3CO-), 3.60-3.82 (2H, m, -CH_2CH-), 4.20-4.40 (1H, m, -CH_2CH-), 4.72-4.85 (1H, m, -CH-NH-), 5.93 (1H,

s, PhCH-), 6.90 (1H, s, Ph_2CH-), 7.25-7.55 (15H, m, aromatic CH); ^1H nmr of crude material had no signal at δ 5.97.

α-R-Mandeloyl-β-acetylamino-L-alanine benzhydryl ester (20b): β-Acetylamino-L-alanine benzhydryl ester (19) (0.022g, 0.070 mmol) was coupled with R-mandelic acid (0.012 g, 1.1 eq.), using 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) (0.020 g, 1.2 eq.) as the coupling agent, and the product was purified as described for the previous experiment, to give the title compound (0.005 g, 16%); R_f 0.3 (20% ethyl acetate / petroleum ether (b.p. 30-40°C)); δ_H (200 MHz, $CDCl_3$) 1.70 (1H, s, -OH), 1.82 (3H, m, CH_3CO-), 3.52-3.82 (2H, m, -CH_2CH-), 4.22-4.35 (1H, m, -CH_2CH-), 4.72-4.85 (1H, m, -CH-NH-), 5.97 (1H, s, PhCH-), 6.90 (1H, s, Ph_2CH-), 7.25-7.55 (15H, m, aromatic CH); ^1H nmr of crude material had no signal at δ 5.93.

α-*N*-*tert*-Butoxycarbonyl-β-amino-L-alanine benzhydryl ester (21): α-*N*-*tert*-Butoxycarbonyl-β-amino-L-alanine (16) (0.145g, 0.7 mmol) and *p*-toluenesulphonic acid (0.149 g, 1.1 eq.) were dissolved in acetonitrile / water (2:1) (10 ml), and the solution was stirred for 10 minutes. Diphenyldiazomethane (0.207 g, 1.5 eq.) was then added, and the resulting solution stirred for 1 hour, during which time the solution was decolourised. The acetonitrile was evaporated *in vacuo*, and the resulting aqueous suspension extracted with ethyl acetate (10 ml). The ethyl acetate solution was washed with saturated $NaHCO_3$ (2 x 10 ml) and water (10 ml), dried (Na_2SO_4), and the ethyl acetate evaporated *in vacuo* to give the title compound as a white solid (0.264 g, quantitative); ν_{max} ($CHCl_3$) 3 420 (w), 3 010 (s), 1 740 (s), 1 705 (s), 1 495 (s), 1 449 (m), 1 367 (m), 1 160 (s), 1 030 (s), 1 008 (s); δ_H (200 MHz, $CDCl_3$) 1.45 (9H, s, $(CH_3)_3C$-), 2.3 (2H, br, -NH_2), 3.05-3.15 (2H, m, -CH_2CH-), 3.78-3.82 (1H, m, -CH_2CH-), 5.42 (1H, br, -NH), 6.92 (1H, s, Ph_2CH-), 7.30-7.48 (10H, m, aromatic CH); *m/z* (DCI (NH_3)) 371 (MH^+, 34%), 339 (72), 313 (20), 297 (43), 168 (33), 167 (100).

α-*N*-(*tert*-Butoxycarbonyl)-β-(3-*tert*-butyldiphenylsilyloxy)-ureido-L-alanine benzhydryl ester (24): A solution of *O*-(*tert*-butyldiphenylsilyl) hydroxylamine (prepared by the method of Bottaro[16]) (0.245 g, 0.90 mmol) in anhydrous dichloromethane (10 ml) was added dropwise over 6 hours, using a syringe pump, to a stirred solution of carbonyl diimidazole (22) (0.147 g, 0.91 mmol) in anhydrous dichloromethane (2 ml). The solution was stirred for a further 12 hours with the formation of a white suspension, then α-*N*-*tert*-butoxycarbonyl-β-amino-L-alanine benzhydryl ester (21) (0.264 g, 0.71 mmol) was added as a solution in anhydrous dichloromethane (5 ml). The resulting yellow solution was stirred for a further 15 hours, then the dichloromethane was evaporated *in vacuo* to give an oil. This was dissolved in ethyl acetate (10 ml), washed with dil. HCl (10 ml), dried (Na_2SO_4), and the ethyl acetate was removed *in vacuo*. The residue was purufied by flash chromatography, eluting with 30% ethyl acetate / petroleum ether (b.p. 30-40°C) to give the title compound as a colourless oil (0.206 g, 43%); R_f 0.3 (30% ethyl acetate / petroleum ether (b.p. 30-40°C)); $[\alpha]_D^{20}$ +5.5 (c 3, $CHCl_3$); ν_{max} ($CHCl_3$) 3 433 (w), 3 022 (s), 2 934 (w), 2 861 (w), 2 361 (m), 2 342 (m), 1 708 (s), 1 531 (m), 1 497 (m), 1 370 (w), 1 162 (s); δ_H (200 MHz, $CDCl_3$) 1.15 (9H, s, $(CH_3)_3C$-Si-), 1.45 (9H, s, $(CH_3)_3C$-O-), 3.52-3.69 (2H, m, -CH_2CH-), 4.37-4.50 (1H, m, -CH_2CH-), 5.38 (1H, d, *J* 10.0 Hz, -CHNH), 6.28 (1H, br, m, -CH_2NH), 6.72 (1H, s, -ONH), 6.90 (Ph_2CH-), 7.35-7.50 and 7.65-7.75 (20H, m, aromatic CH); δ_C (50.3 MHz, $CDCl_3$) 19.1 ($(CH_3)_3$CSi-), 27.1 (($CH_3)_3$CSi-), 28.3 (($CH_3)_3$CO-), 41.2 (-CH_2CH-), 54.4 (-CH_2CH-), 76.2 (Si-Phenyl (*ipso*)), 78.5 (Ph_2CH-), 80.1 ($(CH_3)_3$C-O-), 126.6-139.5 (aromatic), 155.4 (NH-CO_2-), 161.0 (-NHCO-NH-), 169.8 (-$CHCO_2$-); *m/z* (FAB) 668 (MH^+, 4%), 167 (100).

β-(3-Hydroxyureido)-L-alanine (3)[9]: α-*N*-(*tert*-Butoxycarbonyl)-β-(3-*tert*-butyldiphenylsilyloxy)-ureido-L-alanine benzhydryl ester (24) (0.103 g, 0.15 mmol) was dissolved in dil. HCl / dioxan (1:1) (2 ml), and stirred for 2 hours. The dioxan was evaporated *in vacuo* and the aqueous solution was washed with diethyl ether (2 x 2 ml). The aqueous layer was freeze dried to give crude β-(3-hydroxyureido)-(L)-alanine hydrochloride (0.022 g, 74%). This was purified by ion exchange chromatography, using Dowex 50W- X8 resin (100-200 mesh), and eluting with 2N ammonium hydroxide, to give the title compound as a white solid (0.010 g, 41% from α-*N*-(*tert*-butoxycarbonyl)-β-(3-*tert*-butyldiphenylsilyloxy)-ureido-L-alanine benzhydryl ester (24)); $[\alpha]_D^{20}$ -11.5 (c 0.5, 1N HCl) (lit.[9] -12 (c 1.0, 1N HCl)); δ_H (500 MHz, D_2O, HOD suppressed) 3.58-3.60, 3.73-3.77 and 3.95-3.97 (3H, AB-X, *J* 6.5, 2.0, 1.5 Hz, -CH_2CH-); *m/z* (electrospray) 165 (17%), 164 (MH^+, 100%), 131 (62), 126 (30), 122 (39).

1-*O*-(*tert*-Butoxycarbamoyl)-*N*-(*tert*-butoxycarbonyl)-β-chloro-L-alanine (14): 1-Ethyl-3-[3-(dimethylamino)propyl]-carbodiimide (0.133 g, 0.7 mmol) was added to a stirred solution of *N*-(*tert*-butoxycarbonyl)-β-chloro-L-alanine (13) (prepared by the method of Walsh[7]) (0.155 g, 0.7 mmol) and *N*-(*tert*-butoxycarbonyl)-hydroxylamine (prepared by the method of Harris[17]) (0.092 g, 0.7 mmol) in anhydrous dichloromethane (5 ml) and the solution was stirred for 15 hours. The dichloromethane was evaporated *in vacuo* and the resulting oil dissolved in ethyl acetate (10 ml), washed with water (2 x 10 ml), dried (Na_2SO_4), and the ethyl acetate evaporated *in vacuo* to give the title compound as a white solid (0.227 g, 91%). Recrystallisation from ethyl acetate / petroleum ether (b.p. 40-60°C) gave an analytical sample; m.p 121-122°C; R_f 0.1 (10% ethyl acetate / petroleum ether (b.p. 40-60°C)); $[\alpha]_D^{20}$-13.5 (c 1.3, $CHCl_3$). Found: C 46.05, H 6.75, N 8.23; $C_{13}H_{23}ClN_2O_6$ requires C 46.09, H 6.84, N 8.27%; ν_{max} ($CHCl_3$) 2 983 (w), 1 713 (s), 1 504 (m), 1 395 (w), 1 371 (m), 1 253 (w), 1 162 (s); δ_H (200 MHz, $CDCl_3$) 1.46 and 1.50 (2 x 9H, s, (2 x CH_3)$_3$C-), 3.85-4.06 (2H, AB-X, *J* 11.5, 3.5, 4.0 Hz, -CH_2CH-), and 4.86-4.92 (1H, AB-X, -CH_2CH-), 5.46 (1H, d, *J* 8.5 Hz, -NH-C-), 8.09 (1H, s, -NH-O-); δ_C (50.3 MHz, $CDCl_3$) 27.9 and 28.1(2 x (CH_3)$_3$C-), 44.7 (-CH_2CH-), 53.3 (-CH_2CH-), 81.0 and 84.0 (2 x (CH_3)$_3$C-), 155.3 and 155.4 (2 x -NH-CO_2-), 169.3 (-CHCO_2-); *m/z* (CI (NH_3)) 358 (MNH_4^+ (^{37}Cl), 4%), 356 (MNH_4^+ (^{35}Cl), 11), 300 (15), 144 (23), 133 (77), 116 (100).

α-(*N*-*tert*-Butoxycarbonyl)-β-(*N*-*tert*-butoxycarbonyl, *N*-hydroxy)-β-amino-L-alanine (25): Sodium hydride (0.016 g of a 50% suspension in mineral oil, 0.3 mmol, 1.0 eq.) was added to a stirred solution of 1-*O*-(*tert*-butoxycarbamoyl)-*N*-(*tert*-butoxycarbonyl)-β-chloro-L-alanine (14) (0.110 g, 0.3 mmol) in anhydrous dimethylformamide (5 ml) at 0°C, and the suspension was stirred for 1 hour. A solution of Cs_2CO_3 (0.060 g, 0.18 mmol, 0.6 eq.) in water (2 ml) was then added, and the solution was stirred for 10 minutes then washed with diethyl ether (2 x 10 ml), acidified with $KHSO_4$ (1M aq.) and extracted with ethyl acetate (3 x 10 ml). The ethyl acetate fractions were combined, washed with water (3 x 10 ml), dried (Na_2SO_4) and the ethyl acetate evaporated *in vacuo* to give the title compound as a yellow oil (0.093 g, 90%); ν_{max} ($CHCl_3$) 2 983 (w), 1 713 (s), 1 506 (m), 1 394 (m), 1 370 (s), 1 251 (m), 1 161 (s); δ_H (200 MHz, $CDCl_3$) 1.46 and 1.50 (2 x 9H, s, (2 x CH_3)$_3$C-), 3.72-4.05 (2H, m, -CH_2CH-), 4.57-4.89 (1H, m, -CH_2CH-), 5.46 (1H, d, *J* 8.5 Hz, -NH); δ_C (50.3 MHz, $CDCl_3$) 27.3 and 28.2 (2 x (CH_3)$_3$C-), 51.4 (-CH_2CH-), 51.8 (-CH_2CH-), 81.2 and 81.7

(2 x $(CH_3)_3\underline{C}$-), 156.3 and 157.0 (2 x -NH-$\underline{C}O_2$-), 173.4 (-$\underline{C}O_2H$); *m/z* (CI (NH_3)) 320 (MNH_4^+-H_2O, 6%), 264 (18), 251 (27), 151 (55), 135 (51), 133 (49), 116 (94), 95 (76), 79 (100), 74 (94).

α-(*N-tert*-Butoxycarbonyl)-β-(*N-tert*-butoxycarbonyl, *N*-hydroxy)-β-amino-L-alanine benzhydryl ester (26): A solution of diphenyldiazomethane (0.031 g, 0.16 mmol, 1.0 eq.) in acetonitrile (1 ml) was added dropwise to a stirred solution of α-(*N-tert*-butoxycarbonyl)-β-(*N-tert*-butoxycarbonyl, *N*-hydroxy)-β-amino-L-alanine (25) (0.051 g, 0.16 mmol) in acetonitrile (2 ml) until the purple diphenyldiazomethane solution was no longer decolourised on addition. The solution was stirred for a further 1 hour then decolourised by the addition of one drop of acetic acid. The solvent was removed *in vacuo* and the resulting oil purified by flash chromatography, eluting with 10% ethyl acetate / petroleum ether (b.p. 30-40°C) to give the title compound as a colourless oil (0.072 g, 93%); R_f 0.2 (20% ethyl acetate / petroleum ether (b.p. 30-40°C)); $[\alpha]_D^{20}$-2.5 (c 0.85, $CHCl_3$). Found: C 63.90, H 7.18, N 5.68; $C_{26}H_{34}N_2O_7$ requires C 64.18, H 7.04, N 5.76%; ν_{max} ($CHCl_3$) 2 983 (w), 1 719 (s), 1 506 (m), 1 370 (s), 1 250 (s), 1 163 (s); δ_H (200 MHz, $CDCl_3$) 1.44 and 1.46 (2 x 9H, s, 2 x $(CH_3)_3$C-), 3.72-4.01 (2H, AB-X, *J* 14.0, 9.5, 4.0 Hz, -CH_2CH-) and 4.71-4.80 (1H, AB-X, -CH_2CH-), 5.42 (1H, d, *J* 8.0 Hz, -NH), 6.94 (1H, s, Ph_2CH-), 7.32-7.39 (10H, m, aromatic CH), 7.61 (1H, s, -OH); δ_C (50.3 MHz, $CDCl_3$) 27.9 and 28.2 (2 x $(CH_3)_3$C-), 51.47 (-CH_2CH-), 51.5 (-CH_2CH-), 78.7 (Ph_2CH), 81.1 and 81.7 (2 x $(CH_3)_3$C-), 127.2-128.8 (aromatic -CH), 139.4 (aromatic *ipso*), 156.3 and 157.0 (-NH-CO_2-), 169.9 (-CH-CO_2-); *m/z* (FAB (NaOAc)) 509 (MNa^+, 20%), 387 (22), 167 (100).

α-(*N-tert*-Butoxycarbonyl)-β-(*N-tert*-butoxycarbonyl, *N*-propioloxy)-β-amino-L-alanine benzhydryl ester (27): 1,3-Dicyclohexylcarbodiimide (DCC), (0.062 g, 0.30 mmol, 1.1 eq.) was added to a stirred solution of α-(*N-tert*-butoxycarbonyl)-β-(*N-tert*-butoxycarbonyl, *N*-hydroxy)-β-amino-L-alanine benzhydryl ester (26) (0.133 g, 0.27 mmol) and propiolic acid (0.030 g, 0.43 mmol, 1.6 eq.) in anhydrous dichloromethane (5 ml), and the solution was stirred for 18 hours, with the formation of a white suspension in a brown solution. The dichloromethane was evaporated *in vacuo* and the residue suspended in ethyl acetate (2 x 5 ml), filtered, and the filtrate concentrated *in vacuo* to give a brown oil. Purification by flash chromatography, eluting with 30% ethyl acetate / petroleum ether (b.p. 30-40°C) gave the title compound as a white solid (0.132 g, 91%). Recrystallisation from ethyl acetate / petroleum ether (b.p. 40-60°C) gave an analytical sample; m.p. 127-128°C; R_f 0.1 (10% ethyl acetate / petroleum ether (b.p. 30-40°C)); $[\alpha]_D^{20}$ +0.21 (c 1.8, $CHCl_3$). Found: C 64.89, H 6.25, N 5.05; $C_{29}H_{34}N_2O_8$ requires C 64.67, H 6.36, N 5.20%; ν_{max} ($CHCl_3$) 3 300 (br, w), 2 983 (m), 2 128 (m), 1 750 (s), 1 690 (s), 1 498 (m), 1 371 (m), 1 163 (s); δ_H (200 MHz, $CDCl_3$) 1.44 and 1.46 (2 x 9H, s, 2 x $(CH_3)_3$C-), 2.98 (1H, s, -C≡C-H), 3.98-4.23 (2H, AB-X, *J* 15.0, 5.5, 4.5 Hz, -CH_2CH-), and 4.59-4.69 (1H, AB-X, -CH_2CH-), 5.38 (1H, d, *J* 8.0 Hz, -NH), 6.90 (1H, s, Ph_2CH-), 7.13-7.50 (10H, m, aromatic -CH); δ_C (50.3 MHz, $CDCl_3$) 27.8 and 28.2 (2 x $(CH_3)_3$C-), 51.1 (-CHCH_2-), 52.2 (-CH-CH_2), 71.6 (-C≡C-H), 78.6 (Ph_2CH-), 79.8, 80.2, 83.9 (2 x $(CH_3)_3$C- and -C≡CH), 127.3-128.8 (aromatic -CH), 139.7 (aromatic *ipso*), 151.1 (≡C-CO_2N-), 154.5 and 155.4 (2 x -NCO_2-), 169.8 (-CH-CO_2C-); *m/z* (DCI (NH_3)) 556 (MNH_4^+, 9%), 539 (MH^+, 19), 500 (45), 483 (17), 439 (67), 167 (100).

β-(Isoxazolin-5-one-2-yl)-L-alanine (4): α-(*N-tert*-butoxycarbonyl)-β-(*N-tert*-butoxycarbonyl, *N*-propioloxy)-β-amino-L-alanine benzhydryl ester (27) (0.055 g, 0.10 mmol) was dissolved in 98% formic acid (2 ml) and stirred at 35°C for 18 hours. The formic acid was removed *in vacuo* and the residue dissolved in

water (2 ml) and washed with diethyl ether (2 x 2 ml). The aqueous layer was freeze dried to give the title compound as a white solid, (0.015 g, 85%). Reverse phase HPLC (octadecasilane, eluting with 1% formic acid / water) gave an analytical sample; m.p. 178-180°C (decomp.) (lit.[11,18] 178-180°C (decomp.); ν_{max} (KBr disc) 3 500-2 500 (br), 1 705 (s), 1 630-1 590 (s), 1 530 (s); δ_H (200 MHz, D_2O) 3.93 (1H, t, *J* 4.5 Hz, -CH_2C<u>H</u>-), 4.15 (2H, d, *J* 4.5 Hz, -C<u>H</u>$_2$CH-), 5.10 (1H, d, *J* 3.0 Hz, -CH=C<u>H</u>-CO-), 8.18 (1H, d, *J* 3.0 Hz, -C<u>H</u>=CH-CO-); δ_C (50.3 MHz, D_2O) 52.2 (-<u>C</u>H_2CH-), 53.2 (-CH_2<u>C</u>H), 87.6 (-CH=<u>C</u>H-CO-), 154.4 (-<u>C</u>H=CH-CO-), 170.6 and 174.4 (2 x -C=O); *m/z* (electrospray) 173 (MH^+, 100%).

β-(Isoxazolin-5-one-2-yl)-<u>L</u>-alanine benzhydryl ester (28): β-(Isoxazolin-5-one-2-yl)-<u>L</u>-alanine (4) (0.060 g, 0.35 mmol) and *p*-toluene sulphonic acid (0.065 g, 0.34 mmol) were dissolved in acetone / water (2:1) (10 ml) and the solution was stirred for 10 minutes. Diphenyldiazomethane (0.090 g, 0.5 mmol) was then added and the solution stirred for a further 1 hour, during which time the purple solution was decolourised. Water (20 ml) was added, and the solution washed with diethyl ether (20 ml), basified with sat. $NaHCO_3$, and extracted with ethyl acetate (3 x 20 ml). The ethyl acetate layers were combined, dried (Na_2SO_4), and the ethyl acetate evaporated *in vacuo* to give the title compound as a colourless oil (0.080 g, 68%). This was used in the proceeding reactions without further purification. δ_H (200 MHz, $CDCl_3$) 1.70-2.11 (2H, br, -NH_2), 3.91-4.16 (2H, m, -C<u>H</u>$_2$CH-), 3.67-3.79 (1H, m, -CH_2C<u>H</u>-), 5.10 (1H, d, *J* 3.0 Hz, -CH=C<u>H</u>-CO), 6.90 (1H, s, Ph_2C<u>H</u>), 7.30-7.68 (10H, m, aromatic CH), 7.71 (1H, d, *J* 3.0 Hz, -C<u>H</u>=CH-CO).

α-*N*-<u>S</u>-Mandeloyl-β-(Isoxazolin-5-one-2-yl)-<u>L</u>-alanine benzhydryl ester (29): 2-Ethoxy-1-ethoxycarbonyl 1,2-dihydroquinoline (EEDQ) (0.065 g, 0.25 mmol) was added to stirred solution of freshly prepared β-(isoxazolin-5-one-2-yl)-<u>L</u>-alanine benzhydryl ester (28) (0.080 g, 0.24 mmol) and <u>S</u>-mandelic acid (0.04 g, 0.26 mmol) in anhydrous dichloromethane (5 ml), and the reaction mixture stirred for 18 hours. The dichloromethane was evaporated *in vacuo* and the resulting oil dissolved in ethyl acetate (5 ml), washed with dil. HCl (5 ml), sat. $NaHCO_3$ (5 ml) and water (5 ml), dried (Na_2SO_4), and the ethyl acetate evaporated *in vacuo*. The residue was purified by flash chromatography, eluting with 70% ethyl acetate / petroleum ether (b.p. 30-40°C), taking care to combine all fractions that contained either diastereomer of the product, to give the title compound as an oil (0.04 g, 35%); R_f 0.3-0.4 (70% ethyl acetate / petroleum ether (b.p. 30-40°C)); δ_H (partial) (500 MHz, $CDCl_3$) 4.950 (0.9H, d, *J* 3.5 Hz, -N-CH=C<u>H</u>-CO of the diastereomer of (29) corresponding to the <u>L</u>-amino acid (4)), 4.955 (0.1H, d, *J* 3.5 Hz, -N-CH=C<u>H</u>-CO of the diastereomer of (29) corresponding to the <u>D</u>-amino acid (4)), 5.93 (0.9H, s, PhC<u>H</u>(OH)- of the diastereomer of (29) corresponding to the <u>L</u>-amino acid (4)), 5.90(0.1H, s, PhC<u>H</u>(OH)- of the diastereomer of (29) corresponding to the <u>D</u>-amino acid (4)).

***N*-(*tert*-Butoxycarbonyl)-^{15}*N*-hydroxylamine (31)**: A solution of di-*tert*-butyl dicarbonate (1.475 g, 6.8 mmol, 0.95 eq.) in dioxan (10 ml) was added dropwise over 4 hours to a stirred solution of ^{15}*N*-hydroxylamine hydrochloride (0.500 g, 7.1 mmol) in aqueous NaOH (5 ml of a 2N solution). The solution was stirred for a further 18 hours with the formation of a white suspension. The dioxan was evaporated *in vacuo* and the resulting aqueous solution cooled to 0°C, acidified with 1N $KHSO_4$ (1M aq.), and extracted with ethyl acetate (4 x 10 ml). The ethyl acetate fractions were combined, dried (Na_2SO_4), and the ethyl acetate evaporated *in vacuo* to give a 1:1 mixture of *N*-(*tert*-butoxycarbonyl)-^{15}*N*-hydroxylamine (31) and *O*-(*tert*-

butoxycarbonyl)-^{15}N-hydroxylamine (0.640 g, 67%). The mixture was dissolved in 1:1 dioxan / 1N NaOH (10 ml) and hydroxylamine hydrochloride (0.01 g, 3.5% eq.) was added. The solution was stirred for 18 hours before the dioxan was evaporated *in vacuo*, and the resulting aqueous solution cooled to 0°C, acidified with 1N $KHSO_4$ (1M aq.), and extracted with ethyl acetate (4 x 10 ml). The ethyl acetate fractions were combined, dried (Na_2SO_4) and the ethyl acetate evaporated *in vacuo* to give the title compound as a white solid (0.504 g, 53%) (unlabelled *N*-(*tert*-Butoxycarbonyl)-hydroxylamine prepared by this method resulted in 72% yield on an 8 mmol scale); R_f 0.6 (ethyl acetate); δ_H (200 MHz, $CDCl_3$) 1.48 (9H, s, $(CH_3)_3C$-), 6.9 (1H, br, -OH), 7.07 (1H, d, *J* 93.5 Hz, -^{15}NH); δ_C (50.3 MHz, $CDCl_3$) 28.1 ($(\underline{C}H_3)_3C$-), 82.1 ($(CH_3\underline{C}$-), 159.2 (d, *J* 20.5 Hz, -^{15}N-C=O); δ_N (25.3 MHz, $CDCl_3$) 238.0 (d, *J* 94 Hz, -^{15}NH); *m/z* (CI (NH_3)) 152 (MNH_4^+, 33%), 117 (7), 96 (100) (equivalent to unlabelled (31) by R_f, ^{1}H and ^{13}C nmr, and *m/z* .)

1-*O*-(*tert*-Butoxy-^{15}N-carbamoyl)-*N*-(*tert*-butoxycarbonyl)-β-chloro-L-alanine (32): 1-Ethyl-3-[3-(dimethylamino)propyl]-carbodiimide (0.190 g, 1.1 eq.) was added to a stirred solution of *N*-(*tert*-butoxycarbonyl)-^{15}N-hydroxylamine (31) (0.120 g, 0.9 mmol) and *N*-(*tert*-butoxycarbonyl)-β-chloro-L-alanine (13) (0.210 g, 1.05 eq.) in anhydrous dichloromethane (5 ml) and the resulting solution was stirred for 15 hours. The dichloromethane was evaporated *in vacuo* and the residue dissolved in ethyl acetate (10 ml) and washed with water (2 x 10 ml). The ethyl acetate fraction was dried (Na_2SO_4) and the ethyl acetate evaporated *in vacuo* to give the title compound as a white solid (0.298 g, 98%); R_f 0.1 (10% ethyl acetate / petroleum ether (b.p. 30-40°C)); δ_H (200 MHz, $CDCl_3$) 1.47 and 1.51 (2 x 9H, s, 2 x $(CH_3)_3C$-), 3.85-4.07 (2H, AB-X, *J* 11.5, 3.5, 4.0 Hz, -$C\underline{H}_2$CH-), and 4.87-4.93 (1H, AB-X, -$CH_2C\underline{H}$-), 5.42 (1H, d, *J* 8.5 Hz, -NH-C-), 7.90 (1H, d, *J* 85 Hz, -^{15}NH-O-); δ_C (50.3 MHz, $CDCl_3$) 27.9 and 28.1 (2 x $(\underline{C}H_3)_3C$-), 44.7 (-$\underline{C}H_2$CH-), 53.3 (-$CH_2\underline{C}$H-), 81.0 and 83.9 (2 x $(CH_3)_3\underline{C}$-), 155.2 (-NH-CO_2-), 155.5 (-^{15}NH-CO_2-), 169.2 (-$CH\underline{C}O_2$-); δ_N (25.3 MHz, $CDCl_3$) 223.5 (d, *J* 85.5 Hz, -^{15}NH-); *m/z* (CI (NH_3)) 357 (MNH_4^+, 2%), 301 (4), 149 (12), 144 (20), 134 (68), 117 (100), 80 (30).

α-(*N*-*tert*-Butoxycarbonyl)-β-(*^{15}N*-*tert*-butoxycarbonyl, *^{15}N*-hydroxy)-β-*^{15}N*-amino-L-alanine (34): Sodium hydride (0.030 g of a 60% dispersion in mineral oil, 1.0 eq.) was added to a solution of 1-*O*-(*tert*-butoxy-^{15}N-carbamoyl)-*N*-(*tert*-butoxycarbonyl)-β-chloro-L-alanine (32) (0.254 g, 0.75 mmol) in anhydrous dimethylformamide (15 ml) at 0°C, and the suspension was stirred for 1 hour. The resulting isoxazolidin-5-one (33) was hydrolysed *in situ* by the addition of aqueous Cs_2CO_3 (0.125 g in 10 ml H_2O). The solution was stirred for 10 minutes, then washed with diethyl ether (10 ml), acidified with $KHSO_4$ (1M aq.) and extracted into ethyl acetate (4 x 10 ml). The ethyl acetate fractions were combined, washed with water (3 x 10 ml), dried (Na_2SO_4) and the ethyl acetate evaporated *in vacuo* to give the title compound as a yellow oil (0.132 g, 55%), which was used directly in the next reaction without further purification; δ_H (200 MHz, $CDCl_3$) 1.46 and 1.50 (2 x 9H, s, 2 x $(CH_3)_3C$-), 3.72-4.05 (2H, m, -^{15}N-$C\underline{H}_2$CH-), 4.57-4.89 (1H, m, -$CH_2C\underline{H}$-), 5.46 (1H, d, *J* 8.5 Hz, -NH).

α-(*N*-*tert*-Butoxycarbonyl)-β-(*^{15}N*-*tert*-butoxycarbonyl, *^{15}N*-hydroxy)-β-*^{15}N*-amino-L-alanine benzhydryl ester (35): A solution of diphenyldiazomethane (0.072 g, 0.37 mmol, 0.9 eq.) in acetonitrile (1 ml) was added dropwise to a stirred solution of α-(*N*-*tert*-butoxycarbonyl)-β-(*^{15}N*-*tert*-butoxycarbonyl, *^{15}N*-hydroxy)-β-*^{15}N*-amino-L-alanine (34) (0.132 g, 0.41 mmol) in acetonitrile (5 ml) until no further

decolourisation of the purple diphenyldiazomethane solution took place. The acetonitrile was evaporated *in vacuo* and the resulting oil purified by flash chromatography eluting with 10% ethyl acetate / petroleum ether (b.p. 30-40°C) to give the title compound as a colourless oil (0.141 g, 78%); R_f 0.2 (20% ethyl acetate / petroleum ether (b.p. 30-40°C)); δ_H (200 MHz, $CDCl_3$) 1.49 and 1.54 (2 x 9H, s, 2 x $(CH_3)_3C$-), 3.73-4.01 (2H, m, -^{15}N-C$\underline{H}_2$CH-), 4.71-4.79 (1H, m, -^{15}N-CH$_2$C$\underline{H}$-), 5.41 (1H, d, *J* 8.0 Hz, -NH), 6.94 (1H, s, $Ph_2C\underline{H}$-), 7.28-7.41 (10H, m, aromatic CH), 7.61 (1H, s, -OH); δ_C (50.3 MHz, $CDCl_3$) 27.9 and 28.2 (2 x $(\underline{C}H_3)_3C$-), 51.3 (d, *J* 15 Hz, -^{15}N-$\underline{C}H_2$CH-), 51.6 (-^{15}N-CH$_2\underline{C}$H-), 78.7 ($Ph_2\underline{C}H$-), 81.1 and 81.7 (2 x $(CH_3)_3\underline{C}$-), 127.2-128.8 (aromatic -CH), 139.4 (aromatic *ipso*), 156.5 and 157.0 (-NH-CO_2-), 169.9 (-CH-$\underline{C}O_2$-); δ_N (25.3 MHz, $CDCl_3$) 234.5 (s, -^{15}N); *m/z* (FAB (NaOAc)) 510 (MNa^+, 28%), 167 (100).

α–(*N-tert*-Butoxycarbonyl)-β-(*^{15}N-tert*-butoxycarbonyl, *^{15}N*-propioloxy)-β-*^{15}N*-amino-L-alanine benzhydryl ester (36): 1,3-Dicyclohexylcarbodiimide (DCC) (0.085 g, 0.41 mmol, 1.2 eq.) was added to a stirred solution of α–(*N-tert*-butoxycarbonyl)-β-(*^{15}N-tert*-butoxycarbonyl, *^{15}N*-hydroxy)-β-*^{15}N*-amino-L-alanine benzhydryl ester (35) (0.168 g, 0.35 mmol) and propiolic acid (0.027 g, 0.39 mmol, 1.1 eq.) in anhydrous dichloromethane (5 ml), and the reaction mixture was stirred for 15 hours with the formation of a white suspension in a brown solution. The dichloromethane was evaporated *in vacuo* and the resulting solid suspended in ethyl acetate (2 x 5 ml), filtered, and the ethyl acetate evaporated *in vacuo* to give a yellow oil. This was purified by flash chromatography, eluting with 30% ethyl acetate / petroleum ether (b.p. 30-40°C) to give the title compound as a white solid (0.128 g, 68%); R_f 0.1 (10% ethyl acetate / petroleum ether (b.p. 30-40°C)); δ_H (200 MHz, $CDCl_3$) 1.45 and 1.46 (2 x 9H, s, 2 x $(CH_3)_3C$-), 2.99 (1H, s, -C≡C-H), 3.98-4.23 (2H, m, -C$\underline{H}_2$CH-), 4.58-4.70 (1H, m -CH$_2$C$\underline{H}$-), 5.38 (1H, d, *J* 8.0 Hz, -NH), 6.90 (1H, s, $Ph_2C\underline{H}$-), 7.13-7.50 (10H, m, aromatic CH); δ_C (50.3 MHz, $CDCl_3$) 27.8 and 28.2 (2 x $(\underline{C}H_3)_3C$-), 51.1 (d, *J* 7 Hz, -CH$\underline{C}$H$_2$^{15}N-), 52.2 (-$\underline{C}$H-CH$_2$), 71.6 (-C≡$\underline{C}$-H), 78.6 ($Ph_2\underline{C}H$-), 79.8, 80.2, 83.9 (2 x $(CH_3)_3\underline{C}$- and -$\underline{C}$≡CH), 127.3-128.8 (aromatic CH), 139.6 (aromatic *ipso*), 151.0 (≡C-$\underline{C}O_2$^{15}N-), 154.5 (d, *J* 18.5 Hz, -^{15}NHCO_2-), 155.4 (-NHCO_2-), 169.8 (-CH-$\underline{C}O_2$C-); δ_N (25.3 MHz, $CDCl_3$) 214.5 (s, ^{15}N); *m/z* (DCI (NH_3)) 557 (MNH_4^+, 6%), 540 (MH^+, 17), 501 (33), 440 (60), 167 (100).

β-(*^{15}N*-Isoxazolin-5-one-2-yl)-L-alanine (37): α–(*N-tert*-Butoxycarbonyl)-β-(*^{15}N-tert*-butoxycarbonyl-*^{15}N*-propioloxy)-β-*^{15}N*-amino-L-alanine benzhydryl ester (36) (0.051 g, 0.095 mmol) was dissolved in 98% formic acid (1 ml) and stirred at 35°C for 15 hours. The formic acid was evaporated *in vacuo*, and the resulting oil dissolved in water (2 ml) and washed with diethyl ether (2 x 2 ml). The aqueous layer was freeze dried to give the title compound as a brown solid (0.015 g, 91%); δ_H (500 MHz, D_2O) 4.07 (1H, q, *J* 5.0 Hz, -^{15}N-CH$_2$C$\underline{H}$-), 4.28 (2H, d, *J* 5.0 Hz, -^{15}N-C$\underline{H}_2$CH-), 5.24 (1H, dd, *J* 3.5, 4.5 Hz,-^{15}N-CH=C$\underline{H}$-CO-), 8.19, (1H, dd, *J* 3.5, 7.5 Hz, -^{15}N-C$\underline{H}$=CH-CO-); *m/z* (electrospray) 174 (MH^+, 100%), 167 (61), 158 (21).

1-^{13}C-Propiolic acid (40): Butyl lithium (5.0 ml of a 2.18 M solution in hexane, 10.9 mmol) was dissolved in anhydrous THF (10 ml) under an atmosphere of argon in a 50 ml 3-necked flask cooled to 0°C. In a separate 50 ml 2-necked flask, cooled to 0°C, water (10 ml) was added dropwise, using a pressure equalised dropping funnel, onto calcium carbide (10 g), resulting in the evolution of acetylene gas. This was passed through a calcium chloride drying tube, then bubbled through the solution of butyl lithium. The reaction mixture was stirred for 10 minutes with the formation of a white suspension. The mixture and washings (anhydrous THF,

2 x 10 ml) were then transferred, *via* a syringe with a wide bore needle, to a 250 ml flask containing ^{13}C carbon dioxide (10.25 mmol), sealed with a rubber septum. This reaction vessel was then shaken vigorously at room temperature for 1 hour, causing the suspension to turn brown. The reaction was quenched with water (20 ml) and washed with diethyl ether (2 x 10 ml). The THF was removed from the aqueous layer *in vacuo* and the solution acidified (6N HCl) and extracted with diethyl ether (5 x 50 ml). The ether was removed by distillation at atmospheric pressure to give the title compound as a brown semi-solid (0.066 g, 9%); δ_H (200 MHz, $CDCl_3$) 3.01 (1H, d, *J* 5.0 Hz, -$^{13}C\equiv C\underline{H}$).

α–(*N-tert*-Butoxycarbonyl)-β-(*N-tert*-butoxycarbonyl, *N*-(1-^{13}C-propioloxy))-β-amino-L-alanine benzhydryl ester (41): 1,3-Dicyclohexylcarbodiimide (DCC) (0.060 g, 0.29 mmol) was added to a stirred solution of α–(*N-tert*-butoxycarbonyl)-β-(*N-tert*-butoxycarbonyl, *N*-hydroxy)-β-amino-L-alanine benzhydryl ester (26) (0.104 g, 0.21 mmol) and 1-^{13}C-propiolic acid (40) (0.02 g, 0.28 mmol) in anhydrous dichloromethane (4 ml), and the reaction mixture was stirred for 18 hours, with the formation of a white suspension in a brown solution. The dichloromethane was evaporated *in vacuo* and the resulting solid material suspended in ethyl acetate (2 x 5 ml), filtered and the ethyl acetate evaporated *in vacuo* to give a yellow oil. This was purified by flash chromatography, eluting with 30% ethyl acetate / petroleum ether (b.p. 30-40°C) to give the title compound as a white solid (0.036 g, 32%); R_f 0.1 (10% ethyl acetate / petroleum ether (b.p. 30-40°C)); δ_H (200 MHz, $CDCl_3$) 1.44 and 1.46 (2 x 9H, s, 2 x $(CH_3)_3C$-), 2.97 (1H, d, *J* 5.0 Hz, -^{13}C-C≡C-H), 3.98-4.24 (2H, AB-X, *J* 15.0, 5.5, 4.5 Hz, -CH_2CH-), and 4.59-4.68 (1H, AB-X, -$CH_2C\underline{H}$-), 5.35 (1H, d, *J* 8.5 Hz, -NH), 6.90 (1H, s, $Ph_2C\underline{H}$-), 7.16-7.50 (10H, m, aromatic CH); δ_C (50.3 MHz, $CDCl_3$) 27.8 and 28.2 (2 x $(\underline{C}H_3)_3C$-), 51.1 (-CH$\underline{C}H_2$-), 52.3 (-$\underline{C}$H-CH_2), 71.6 (-C≡$\underline{C}$-H), 78.6 ($Ph_2\underline{C}$H-), 79.8, 80.3, 83.9 (2 x $(CH_3)_3\underline{C}$- and -$\underline{C}$≡CH), 127.3-128.8 (aromatic -CH), 139.7 (aromatic *ipso*), 151.0 (≡C-$^{13}\underline{C}O_2$N-), 154.5 and 155.4 (2 x -NCO_2-), 169.8 (-CH-$\underline{C}O_2$C-); *m/z* (DCI (NH_3)) 540 (MH^+, 3%), 501 (12), 440 (21), 167 (100).

β-(5-^{13}C-Isoxazolin-5-one-2-yl)-L-alanine (42): α–(*N-tert*-Butoxycarbonyl)-β-(*N-tert*-butoxycarbonyl-*N*-1-^{13}C-propioloxy)-β-amino-L-alanine benzhydryl ester (41) (0.043 g, 0.08 mmol) was dissolved in 98% formic acid (1 ml) and the solution stirred at 35°C for 15 hours. The formic acid was evaporated *in vacuo*, and the resulting oil dissolved in water (2 ml) and washed with diethyl ether (2 x 2 ml). The aqueous layer was freeze dried to give the title compound as a brown solid (0.013 g, 94%); δ_H (200 MHz, D_2O) 3.96 (1H, t, *J* 5.0 Hz, -$CH_2C\underline{H}$-), 4.17 (2H, d, *J* 5.0 Hz, -$C\underline{H}_2$CH-), 5.12 (1H, dd, *J* 3.5, 7.5 Hz, -CH=C$\underline{H}$-^{13}CO-), 8.08 (1H, dd, *J* 3.5, 9.5 Hz, -C$\underline{H}$=CH-^{13}CO-); δ_C (50.3 MHz, D_2O) 52.2 (-$\underline{C}H_2$CH-), 53.2 (-$CH_2\underline{C}$H), 87.6 (-CH=$\underline{C}$H-CO-), 154.4 (-$\underline{C}$H=CH-CO-), 170.6 (-C=O), 174.7 (-^{13}C=O); *m/z* (electrospray) 174 (MH^+, 100%), 167 (91), 158 (27), 147 (38).

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